CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
PROHEXADIONE CALCIUM

Chemical Code # 005497, Tolerance # 52590

Original: 1/14/00
Revised: 4/5/00

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, no adverse effect.
Chronic toxicity, dog:	No data gap, no adverse effect.
Oncogenicity, mouse:	No data gap, no adverse effect.
Reproduction, rat:	No data gap, no adverse effect.
Teratology, rat:	No data gap, no adverse effect.
Teratology, rabbit:	No data gap, no adverse effect.
Gene mutation:	No data gap, no adverse effect.
Chromosome effects:	No data gap, possible adverse effect.
DNA damage:	No data gap, no adverse effect.
Neurotoxicity:	Not required at this time[1]

Toxicology one-liners are attached.
All record numbers through 166699 were examined.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.

File name: T000405
Original: t000114

[1]Acute and subchronic neurotoxicity studies were submitted and no adverse effects were detected.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

COMBINED, RAT

** 018 166675 ABX-112 Technical: Chronic Feeding and Oncogenicity Study in Rats,@ (Inoue, H.; Biosafety Research Center; Foods, Drugs & Pesticides (An-Pyo Center), Shizuoka, Japan; An-Pyo Center report #: 2421; BASF Registration Document #: 93/10141; 2/17/93). BX-112 (92.8 - 93.5% pure) was fed in diet to Fischer F344 rats (SPF) at 0, 400, 2000, 10000 & 20000 ppm (50/sex/dose--main group & 30/sex/dose satellite group) for 104 weeks. Average BX-112 intake was18.5, 93.9, 469 & 968 mg/kg in males and 22.3, 114, 572 & 1180 mg/kg for females. **Chronic NOEL = 2000 ppm** (Both sexes at 20000 ppm showed significantly decreased absolute bodyweights and gains (primarily in the first year). Both sexes, primarily at 20000 ppm, showed significantly increased food consumption, intermittently, throughout the study but primarily in the first year. Food efficiency was decreased intermittently in both sexes, primarily at $\geq$ 10000 ppm, throughout the study. Hematology and blood chemistry effects were observed in both sexes at 20000 ppm at weeks 26, 52, 78 and 104 weeks. Both sexes at $\geq$ 10000 ppm (week 26) and males at 20000 ppm (weeks 52 & 104) had urinalysis effects. Kidney weights (absolute and/or relative) were increased in both sexes throughout the study and liver weights were decreased throughout the study in males at 20000 ppm. Males had decreased testes weights at 20000 ppm at week 104. Histopathology was primarily in the stomach at both 52 and 104 weeks in both sexes at 20000 ppm (hyperkeratosis, squamous cell hyperplasia, basal cell hyperplasia). **Oncogenic NOEL > 20000 ppm** (No treatment-related oncogenic effects were observed at any dose). No adverse effect. Acceptable. M. Silva, 12/3/99.

CHRONIC TOXICITY, DOG

** 019 166676 ABX-112 Toxicity Study in Beagle Dogs (Final Report - Repeated Daily Dosage for 52 Weeks),@ (Wrench, S.M., McLean, T.A., Buist, D.P., Crook, D., Majeed, S.K. and Gopinath, C.; Huntingdon Research Centre, Ltd.; Cambridgeshire, UK; HRC Report #: KCI 40/911211; BASF Registration Document #: 92/11133; 2/3/97). BX-112 (93.8% & 91.9% pure) was administered orally (capsule) to Beagle dogs (4/sex/dose) at 0, 20, 200 and 1000 mg/kg/day. MCV was significantly increased in both sexes (primarily males at $\geq$ 200 mg/kg) at 1000 mg/kg throughout the test. PVC, Hb and RBCs were decreased in both sexes at 1000 mg/kg. Protein (total and albumen) was decreased in both sexes at $\geq$ 200 mg/kg throughout the study. Potassium was decreased and Cl was increased in both sexes at $\geq$ 200 mg/kg. Specific gravity of urine was significantly decreased, volume and pH were significantly increased in both sexes at 1000 mg/kg. There were significantly decreased thymus weights at $\geq$ 200 mg/kg and significantly increased (adjusted for bodyweight) lung weights in females at 1000 mg/kg. Both sexes had increased incidence in dilated basophilic cortical tubules and fibrosis in the kidneys at $\geq$ 200 mg/kg (M: 2/4 at 200 mg/kg & 1/4 at 1000 mg/kg; F: 1/4 at 200 mg/kg & 1/4 at 1000 mg/kg). Dilated cortical tubules were increased at $\geq$ 200 mg/kg (M: 1/4 at 200 mg/kg & 2/4 at 1000 mg/kg; F: 1/4 at 1000 mg/kg). NOEL = 20 mg/kg No adverse effect. Acceptable. M. Silva, 1/14/00.

ONCOGENICITY, MOUSE

** 020 166678 ABX-112 Technical: Oncogenicity Study in Mice,@ (Inoue, H.; Biosafety Research Center; Foods, Drugs & Pesticides (An-Pyo Center), Shizuoka, Japan; An-Pyo Center Report #: 2313; BASF Registration Document #: 92/11557; 12/2/92). BX-112 (92.8% - 93.5% pure--4 lots used) was fed in diet to SPF B6C3F1 mice (70/sex/dose total) for 52 weeks (10/sex/dose), 78 weeks (10/sex/dose) and 104 weeks (50/sex/dose) at nominal concentrations of 0, 400, 2000, 20000 and 40000 ppm. Systemic NOEL = 2000 ppm (males: 279 mg/kg/day; females: 351 mg/kg/day) (There was significantly decreased body weight at ≥ 20000 ppm in both sexes intermittently throughout the study. Food consumption was significantly increased at ≥ 20000 ppm intermittently throughout the study in both sexes but was significantly increased in males at 40000 ppm (over 104 weeks). Food efficiency was significantly decreased in males at ≥ 20000 ppm and in females at 40000 ppm over 104 weeks. Males at ≥ 20000 ppm had increased HCT% and decreased MCHC% (week 52). HCT%, RBC (females) was increased at ≥ 20000 ppm and HGB (males ≥ 20000 ppm; females 40000 ppm), MCH and MCHC% (males) at 40000 ppm were increased (week 78). HCT% was increased in females at ≥ 20000 ppm (week 104). Relative (to body) at 104 weeks, brain (males ≥ 20000 ppm; females 40000 ppm), liver (males ≥ 20000 ppm), testes (≥ 20000 ppm), ovary (40000 ppm) and kidney weights (males 40000 ppm) were increased. At week 78, the gross findings showed decreased liver nodule formation in males at 40000 ppm (5/10, 3/9, 4/10, 5/10, 1/10 at 0, 400, 2000, 20000 and 40000 ppm, respectively). Gross pathology showed increased incidence of enlarged spleen and decreased white patch/zone in forestomach and liver at 40000 ppm at 104 weeks. Histopathologically there was an increase in both sexes in stomach ectopic tissue, hyperkeratosis and squamous cell hyperplasia at 40000 ppm (104 weeks). Kidney glomerulosclerosis was decreased at week 104 at 40000 ppm.) Oncogenicity NOEL > 40000 ppm; M: 5,911 mg/kg/day & F: 7,334 mg/kg/day (There were no increases in oncogenicity in treated groups, compared to controls.) This study is acceptable with no adverse effects. M. Silva, 12/7/99.

REPRODUCTION, RAT

023 166687 AReproductive Range-Finding Study in Mated Rats With BX-112,@ (York, R.G., International Research and Development Corporation, Mattawan, MI; Laboratory Project ID#: 442-035; BASF Registration Document #: 90/10502; 8/9/90). BX-112 (93.3% pure) was fed in diet to Charles River Crl:CD VAF/Plus**7** rats (8/sex/dose) at 0, 400, 2000, 10000 and 50000 ppm from 28 days prior to mating, through weaning of F1 pups. Systemic NOEL = 10000 ppm (At necropsy, thickened stomach mucosa was observed for 3/8 males at 50000 ppm. Body weights of both sexes were transitionally decreased at 50000 ppm during the first 1 - 3 weeks on study. Throughout the lactation period, body weights of the dams at 50000 ppm were increased. Food consumption (g/kg/day) was increased in males during weeks 3 - 4 at 50000 ppm. Females consumed significantly more than controls (g/kg/day) from gestation day 7 - 14 and they continued to consume more throughout gestation at 50000 ppm.) Reproduction NOEL = 50000 ppm (No effects at any dose.) Pup NOEL = 10000 ppm (Pup weights were lower at 50000 ppm on lactation days 7, 14 and 21.) No adverse effects. These data are supplemental. M. Silva, 12/10/99.

** 024 166688 ATwo Generation Dietary Reproduction Study in Rats with BX-112,@ (York, R.G.; International Research and Development Corporation, Mattawan, MI; Laboratory Project ID #: 442-037; BASF Registration Document #: 92/11170; 10/6/92). BX-112 (91.9 - 93.8% pure) was fed in diet to Charles River Crl:CD VAF/Plus**7** (26/sex/dose) at 0, 500, 5000 and 50000 ppm from premating (56 days) of F0 parents through weaning of F2 pups (continuous feeding through 2 generations of breeding). Parental Systemic NOEL = 500 ppm (F0 at 5000 ppm had 2M & at 50000 ppm, 2M & 1F died or were killed *in extremis*. F0 dam body weights at 50000 ppm were decreased during gestation and lactation. F1 males had decreased mean body weights at 50000 ppm. In the premating weeks, mean weekly body weights were decreased in F1 females at ≥ 5000 ppm. Mean body weights were decreased on lactation day 7 (50000 ppm) and on lactation day 21 (≥ 5000 ppm). Initially F1 parental generation (post-weaning, both sexes) showed reduced mean weekly food consumption at 50000 ppm.

During F1 parental generation for gestation days (gd) 6 - 15 & 15 - 20, food consumption at 50000 ppm was increased. F0 (both sexes) had increased mean water consumption (F also at gestation & lactation) at 50000 ppm. F1 parental generation showed increased mean water consumption for females at 50000 ppm throughout most of the study. At 50000 ppm, F0 males showed 10/24 with dark red foci/focus in glandular stomach & thickened limiting ridge in nonglandular stomach in 9/24. Females at 50000 ppm showed thickening in the nonglandular stomach (9/26 at 5000 ppm & 16/25 at 50000 ppm). Macroscopically, males showed increased thickening of the limiting ridge in the nonglandular stomach (2/26, 500 ppm; 1/26, 5000 ppm; 23/26, 50000 ppm) and females showed increased thickening of the limiting ridge of the nonglandular stomach (25/26). Histopathologically, F1 males had increased glandular stomach atrophy, dysplasia, edema, lymphocytic infiltration & trace metaplasia and hypertrophic hyperstaining gastric cells at 50000 ppm. F1 males had increased nonglandular stomach hyperkeratosis, acanthosis at the limiting ridge and keratin cysts at all doses (dose-related increase). F1 females had a slight increase in kidney effects at 50000 ppm (cyst, hyaline cast, tubular dilatation, fibrosis, hydronephrosis, lymphocytic infiltration, mineralization, tubular regeneneration) and in glandular stomach atrophy, dysplasia, erosion, metaplasia, neutrophil infiltration & hypertrophic hyperstaining gastric cells. F1 females had an increase in nonglandular stomach acanthosis at 50000 ppm and hyperkeratosis & acanthosis at the limiting ridge at $\geq$ 5000 ppm.) Pup NOEL = 5000 ppm (Observations showed 14 (7.9%) of F2 offspring were pale in color at 50000 ppm & lower pup bodyweight--20% day 21.) Reproduction NOEL > 50000 ppm (No reproductive effects at any dose.) Acceptable. No adverse effects. M. Silva, 12/15/99.

TERATOLOGY, RAT

021 166680 ARangefinding Teratology Study in Rats with BX-112,@ (Schardein, J.L.; International Research and Development Corporation, Tokyo, Japan; Laboratory Poject ID: 442-026; BASF Registration Document #: 89/10342; 4/4/89). BX-112 (89.8% pure) was administered by gavage to mated Sprague-Dawley derived Charles River COBS**7** CD**7** rats (6/dose) at 0 (0.5% carboxymethyl cellulose), 100, 250, 500 and 1000 mg/kg/day (limit test) from days 6 through15 of gestation (evidence of mating = gestation day 0). Maternal NOEL > 1000 mg/kg (No treatment-related effects at any dose.) Developmental NOEL > 1000 mg/kg (No treatment-related effects at any dose.) No adverse effects. These data are supplemental. M. Silva, 12/17/99

** 021 166682 ATeratology Study of BX-112 in Rats,@ (Schardein, J.L.; International Research and Development Corporation, Mattawan, MI; Laboratory Project ID #: 442-027; BASF Registration Document #: 90/10503; 8/9/90). BX-112 (89.8% pure) was administered by gavage to Sprague-Dawley derived Charles River COBS**7** CD**7** (25/dose) at 0 (0.5% carboxymethyl cellulose), 100, 300 and 1000 mg/kg (limit test) during gestation days 6 through 15 (10 ml/kg volume). Maternal NOEL > 1000 mg/kg (No treatment-related effects at any dose.) Developmental NOEL > 1000 mg/kg (No treatment-related effects at any dose.) No adverse effect. Acceptable. M. Silva, 12/21/99.

TERATOLOGY, RABBIT

022 166683 ARangefinding Teratology Study in Rabbits with BX-112,@ (Schardein, J.L.; International Research and Development Corporation, Mattawan, MI; Laboratory Project ID: 442-028; BASF Registration Document #: 89/10343; 5/18/89). BX-112 (89.8% pure) was administered by gavage to artificially inseminated New Zealand White SPF rabbits (6/dose) at 0 (0.5% carboxymethyl cellulose), 100, 250, 500 and 1000 mg/kg/day (limit test) from days 7 through19 of gestation (day of insemination = gestation day 0). Maternal NOEL = 100 mg/kg (Two animals at 500 and 3 at 1000 mg/kg died or were sacrificed *in extremis* and decreased weight gain. Lung congestion and gastric irritation (discoloration & hemorrhaging) were noted in animals that died at $\geq$ 500 mg/kg. There was an increase in abortions at $\geq$ 500 mg/kg at 1000 mg/kg, inflammatory liver and lung foci and stomach erosion were also observed.

Ano-genital staining, labored breathing, impaired mobility, gasping, increased salivation and moribundity were observed at ≥ 500 mg/kg. Additional treatment-related signs were at 1000 mg/kg. During dosing, decreased weight gains at 250 and 500 mg/kg and weight loss at 1000 mg/kg were observed. Overall from gestation day 0 to 29, body weight was decreased at 1000 mg/kg. There was an increase in postimplantation loss at 1000 mg/kg resulting in fewer viable fetuses/doe. Developmental NOEL > 1000 mg/kg (No treatment-related effects at any dose.) No adverse developmental effects. These data are supplemental. M. Silva, 12/17/99

022 166684 ATeratology Study in Rabbits with BX-112,@ (York, R.G.; International Research and Development Corporation, Mattawan, MI; Laboratory Project ID: 442-029; BASF Registration Document #: 90/10504; 3/30/90). BX-112 (89.8% & 93.2% pure) was administered by gavage to artificially inseminated New Zealand White SPF rabbits (20/dose) at 0 (0.5% carboxymethyl cellulose), 40, 200 and 750 mg/kg/day in 3 ml/kg from days 7 through 19 of gestation (day of insemination = gestation day 0). Maternal NOEL = 40 mg/kg (Death and clinical observations were increased at ≥ 200 mg/kg. Body weights were decreased at ≥ 200 mg/kg. The mean postimplantation loss at 750 mg/kg was slightly elevated compared to controls.) Developmental NOEL > 750 mg/kg (No treatment-related effects at any dose.) Not acceptable and not upgradeable, since there were too few dams at ≥ 200 mg/kg to assess developmental effects (9 at 200 mg/kg/day and 2 at 750 mg/kg/day). M. Silva, 12/17/99

021 166680 ARange-finding Teratology Study in Rats with BX-112,@ (Schardein, J.L.; International Research and Development Corporation; Laboratory Poject ID: 442-026; BASF Registration Document #: 89/10342; 4/4/89). BX-112 (89.8% pure) was administered by gavage to mated Sprague-Dawley derived Charles River COBS**7** CD**7** rats (6/dose) at 0 (0.5% carboxymethyl cellulose), 100, 250, 500 and 1000 mg/kg/day (limit test) from days 6 through15 of gestation (evidence of mating = gestation day 0). Maternal NOEL > 1000 mg/kg (No treatment-related effects at any dose.) Developmental NOEL > 1000 mg/kg (No treatment-related effects at any dose.) No adverse effects. These data are supplemental. M. Silva, 12/17/99

022 166685 ATeratology Study in Rabbits with BX-112,@ (York, R.G.; International Research and Development Corporation, Mattawan, MI; Laboratory Project ID: 442-039; BASF Registration Document #: 91/11377; 9/10/91). BX-112 (92.8% pure) was administered by gavage to artificially inseminated New Zealand White SPF rabbits (20/dose) at 0 (0.5% carboxymethyl cellulose), 30, 75, and 150 mg/kg/day in 3 ml/kg from days 7 through19 of gestation (day of insemination = gestation day 0). Maternal NOEL < 30 mg/kg (Deaths occurred at all treatment levels due to unknown causes.) Developmental NOEL = 150 mg/kg (No treatment-related effects at any dose.) This study is not acceptable and not upgradeable since a maternal NOEL was not achieved. No adverse developmental effects were observed. M. Silva, 1/3/00.

** 022 166686 A BX-112: Teratology Study in the Rabbits,@ (Kawanishi, H.; Toxicology Research Center; Imamichi Institute for Animal Reproduction, Ibaraki, Japan; Laboratory Project ID: 275; BASF Registration Document #: 92/11169; 7/7/92). BX-112 (91.9% pure) was administered by gavage to pregnant (mated) New Zealand White SPF rabbits (18/dose) at 0 (0.5% carboxymethyl cellulose), 30, 100 and 350 mg/kg/day from days 6 through18 of gestation (day of copulation = gestation day 0). Maternal NOEL = 100 mg/kg (Abortions occurred at 350 mg/kg.) Developmental NOEL = 350 mg/kg (No treatment-related effects at any dose.) No adverse developmental effects were observed. Acceptable. M. Silva, 1/4/00.

GENE MUTATION

** 025 166689 ABacterial Reverse Gene Mutation Assay of BX-112 Technical,@ (Jones, E. & Wilson, L.A.; Huntingdon Research Centre, Ltd.; Cambridgeshire, UK; HRC Project #: KCI 25/881559; BASF Registration Document #: 91/10807; 2/6/97). BX-112 (89.8% pure) was added to bacterial cultures (*Salmonella typhimurium*: TA1535, TA1537, TA1538, TA98, TA100 and *Escherichia coli* strain WP2

uvrA) at 0, 5, 50, 500 and 5000 ug/plate (dose rangefinding test) and 312.5, 625, 1250, 2500 and 5000 ug/plate (mutation test) in a reverse mutation assay. Cultures (in triplicate; +/- S9), were maintained for 72 hours after treatment with BX-112. A repeat test was also performed. The dose rangefinding study showed there was no toxicity of BX-112 to the tester strains. Therefore, 5000 ug/plate was chosen as the top dose level in the mutation tests. Results of the definitive tests showed no increase in revertant colonies with increased dose of BX-112 (up to 5000 ug/plate) either with or without S9. The positive controls functioned as expected. Acceptable, with no adverse effects. M. Silva, 1/5/00.

** 025 166690 A*B. Subtilis* Assay to Determine the Potential of BX-112 Technical to Damage DNA,@ (Jones, E. & Wilson, L.A.; Huntingdon Research Centre, Ltd.; Cambridgeshire, UK; HRC Project #: KCI 26/881489; BASF Registration Document #: 89/10344; 2/6/97). BX-112 (89.8% pure) was added to bacterial cultures (*Bacillus Subtilis* strains: H17 (rec+) & M45 (rec-)) at 0, 50, 150, 500, 1500 and 5000 ug/disk (spot test) and 0, 50, 150, 500, 1500 and 5000 ug/ml (DNA repair test). For the spot test, single plates (+/- S9), were treated for 18 - 20 hours with BX-112. For the definitive test, preincubated (37 ^{o}C for 1 hour) treated mixtures were cultured for 24 hours (duplicate plates/dose; +/- S9). The definitive test was repeated. BX-112 zone diameters obtained in the spot test did not show toxicity towards either of the tester strains. A top dose level of 5000 ug/ml was chosen for the main tests. For the definitive DNA repair tests there was no evidence of ability to cause damage to DNA in this system, as determined by the survival index. The positive controls functioned as expected. Acceptable, with no adverse effects. M. Silva, 1/5/00.

** 025 166691 AMouse Micronucleus Test on BX-112,@ (Proudlock, R.J., Haynes, P., Meaking, K.; Huntingdon Research Centre, Ltd.; Cambridgeshire, UK; HRC Project #: KCI 28/881641; BASF Registration Document #: 91/10808; 2/6/97). BX-112 (89.8% pure) was administered by gavage to CD-1 SPF Swiss mice in a single dose for a preliminary toxicity test at 0, 1000, 2000, 3500, 5000 and 6000 mg/kg (2/sex/dose), followed by a 72 hour observation period, after which they were sacrificed. The definitive study was performed at 0, 1250, 2500 and 5000 mg/kg (5/sex/dose/timepoint + 2 additional mice at 5000 mg/kg, in case of death) and mice were sacrificed at 24, 48 and 72 hours. The positive control was Mitomycin C (12 mg/kg) and the positive control mice were terminated at 24 hours. Minor toxicity was observed at 5000 and 6000 mg/kg in the preliminary test. There was slight pilo-erection and slight hunched posture observed for up to 2 hours postdosing. In the definitive study, slight pilo-erection and slight hunched posture were observed in animals treated at $\geq$ 2500 mg/kg (for 2 - 4 hours postdosing). At 5000 mg/kg, lethargy was observed for 2 - 4 hours postdosing. There were no significant increases in the number of micronucleated polychromatic erythrocytes at any time point. There were no increases in incidence of micronucleated normochromatic erythrocytes at any time point or dose. There were no decreases in the ratio of polychromatic to normochromatic erythrocytes. The positive control functioned as expected. Acceptable, with no adverse effects. M. Silva, 1/6/00.

** 025 166692 AGene Mutation Assay in Chinese Hamster V79 Cells *In Vitro* with Prohexadione-Calcium,@ (Mullerschon, H.; Cytotest Cell Research GambH & Co., KG, FRG; CCR Project #: 304918; BASF Project #: 50M0148/929029; BASF Registration Document #: 92/11328; 10/20/92). BX-112 (93.3% pure) was used on Chinese hamster V79 cells in duplicate cultures at 0, 50, 100, 250 and 500 ug/ml (no S9) and 0, 50, 100, 250 and 475 ug/ml (+S9) in an HGPRT locus test. The experiment was repeated. A preliminary plating efficiency assay (duplicate plates) was performed at 0, 0.10, 0.30, 1, 3, 10, 30, 100, 250 and 500 ug/ml (no S9) and 0, 0.10, 0.30, 1, 3, 10, 30, 100, 250 and 475 ug/ml (+S9). There were no toxic effects up to 500 ug/ml of BX-112 (no S9), where there was a 51% decreased relative plating efficiency with EMS. There was a 23% decreased plating efficiency at 475 ug/ml BX-112 (+ S9), in trial I but none in trial II. In the definitive study, there was no increase in mutation with treatment of BX-112. Acceptable, with no adverse effects. M. Silva, 1/7/00.

CHROMOSOME EFFECTS

**** 025 166693** AAnalysis of Metaphase Chromosomes Obtained From CHO Cultured In Vitro and Treated With BX-112,@ (Brooker, P.C., Gray, V.M., Akhurst, L. C. and Cavaliere, C.; Huntingdon Research Centre, Ltd.; Cambridgeshire, UK; HRC Project #: KCI 27/89678; 2/3/97). BX-112 (89.8% pure) was used on a Chinese hamster ovary cell line (K_1-BH_4) at 0 (4 replicates), 124, 250 and 500 ug/ml (2 replicates/dose/set; sets 1 - 3) and at 62.5, 125 and 250 ug/ml (4th set). Sets 1 & 2: 6 hr treatment (+S9 and no S9), harvest 24 hr; Set 3: 24 hour treatment (no S9), harvest at 24 hr; Set 4: 48 hr treatment (no S9), 48 hr harvest. There was no increase in chromosome aberrations. There was, however, an increased incidence in polyploidy (defined as 27 or more chromosomes) at 500 ug/ml in an initial and a repeat study (no S9), which was outside the historical controls. Possible adverse effect. Acceptable. M. Silva, 1/7/00.

** 025 166694 ABX-112 Analysis of Structural and Numerical Chromosome Aberrations in Rat Bone Marrow,@ (Proudlock, R.J., Haynes, P. and Howell, A.; Huntingdon Research Centre, Ltd.; Cambridgeshire, UK; HRC Project #: KCI 44/901498; BASF Report #: 90/10505; 2/6/97). BX-112 (93.8% pure) was administered in a single gavage dose to CD® Crl:COBS Sprague-Dawley rats (5/sex/dose/time point) at 5000 mg/kg for 6, 24 or 48 hours prior to termination. BX-112 did not induce chromosome damage or polyploidy/aneuploidy in rat bone marrow cells when administered orally in this test. Acceptable. No adverse effect. M. Silva, 1/12/00.

DNA DAMAGE

** 025 166695 AUnscheduled DNA Synthesis in Primary Hepatocytes of Male Rats In Vitro With Prohexadione-Calcium,@ (Fautz, R.; Cytotest Cell Research GambH & Co., KG, FRG; CCR Project #: 304920; BASF-Project #: 80M0148/929030; BASF Registration Document #: 92/11633; 12/3/92). BX-112 (93.3% pure) was used on Wistar/WU male primary rat hepatocytes at 0, 0.5, 1.67, 3.33, 5.0, 16.67, 33.33, 50, 166.67, 333.33 and 500 ug/ml (pre-experiment toxicity; 2 experiments) or at 5.0, 16.67, 50, 166.67 and 500 ug/ml (triplicate plating; 2 experiments). Cells were exposed to BX-112 for 18 hours in the presence of ^{3}HTdR for both the preliminary toxicity and the definitive tests. BX-112 precipitated in culture at 333.33 and 500 ug/ml. At 500 ug/ml there was toxicity by reduction of uptake of neutral red. BX-112 did not induce DNA-damage leading to repair synthesis in this test system. Acceptable. M. Silva, 1/12/00.

NEUROTOXICITY

012; 166667; "Report: Prohexadione-Calcium-Acute Oral Neurotoxicity Study in Wistar Rats" (Mellert, W. et al., Department of Toxicology, BASF Aktiengesellschaft, Rhein, Germany, Laboratory Project Identification 20C0277/94018, 8/29/96). 818. Prohexadione-Calcium (Batch No. G14-37-114, purity=92%), diluted in 0.5% aqueous carboxymethyl cellulose solution, was administered by gavage in a single dose to 10 Wistar rats per sex per dose at dose levels of 0 (vehicle only), 500, 1000, and 2000 mg/kg. No animals died. No treatment-related clinical signs were observed. No treatment-related effects were observed during FOB assessments. No dose-related effects were observed in overall motor activity assessments. Macroscopic and microscopic examinations revealed no treatment-related abnormalities. **No adverse effects**. NOEL (M/F)=2000 mg/kg (based on no effects at HDT). **Acceptable.** (Corlett and Leung, 2/7/00)

017; 166674; "Report: Prohexadione Calcium-Subchronic Oral Dietary Neurotoxicity Study in Wistar Rats" (Mellert, W. et al., Department of Toxicology, BASF Aktiengesellschaft, Rhein, Germany, Laboratory Project Identification 50C0277/94016, BASF Registration Document No. 96/10816, 8/29/96). 827. Prohexadione-Calcium - Technical (Batch No. G14-37-114, purity=92%) was admixed to the feed at dose

levels of 0 (diet), 1000, 4000, or 16000 ppm (0, 70, 285, and 1148 mg/kg/day, respectively, for males and 0, 85, 330, and 1348 mg/kg/day, respectively, for females) and fed to 10 Wistar rats per sex per dose level for 3 months. No animals died. No treatment-related clinical signs were observed. No treatment-related effects were observed during FOB assessments. No treatment-related effects on the total number of beam interrupts were observed during motor activity assessments. Macroscopic and microscopic examinations revealed no treatment-related abnormalities. **No adverse effects.** NOEL (M)=1148 mg/kg/day (16000 ppm) and (F)=1348 mg/kg/day (16000 ppm) (based on no effects at HDT). **Acceptable.** (Corlett and Leung, 2/18/00)

SUBCHRONIC STUDIES

(Oral)

013; 166668; "A Four Week Dietary Range-Finding Study in Rats with BX-112 (Prohexadione-Calcium) Technical" (Inoue, H., Biosafety Research Center, Food, Drugs, and Pesticides (An-Pyo Center), Shizuoka-ken, Japan, BASF Registration Document No. 91/10806, 11/5/88). Technical Prohexadione Calcium (BX-112 Technical) (Lot No. G14-03, purity=92.1%) was admixed to the feed at dose levels of 0, 300, 1000, 3000, or 10000 ppm (0, 30.0, 101, 301, and 1031 mg/kg/day, respectively, for males and 0, 30.3, 102, 310, and 1024 mg/kg/day, respectively, for females) and fed to 10 Fischer 344 rats per sex per dose level for 4 weeks. No animals died. No treatment-related clinical signs were observed. Statistically significant decreases in mean red blood cell, hemoglobin, and hematocrit levels in males at 10000 ppm were observed. Macroscopic and microscopic examinations revealed no treatment-related abnormalities. **No adverse effects indicated.** NOEL (M/F)=1031 mg/kg/day (10000 ppm), (F)=1024 mg/kg/day (10000 ppm) (based on no effects at HDT). **Supplemental** (no ophthalmological examinations conducted and animals exposed to the test article for only 4 weeks). (Corlett, 2/9/00)

014; 166669; "Three Month Subchronic Feeding Study in Rats with BX-112 Technical" (Inoue, H., Biosafety Research Center, Food, Drugs, and Pesticides (An-Pyo Center), Shizuoka-ken, Japan, An-Pyo Center Report No. 2012, BASF Registration Document No. 91/11376, 12/11/91). Technical BX-112 (Lot No. G14-06, purity=93.2%) was admixed to the feed at dose levels of 0 (basal diet only), 1000, 10000, 30000, or 50000 ppm (0, 73.1, 734, 2270, and 3924 mg/kg/day, respectively, for males and 0, 80.4, 815, 2478, and 4221 mg/kg/day, respectively, for females) and fed to 10 F344 (Fischer) rats per sex per dose level for 3 months. No animals died. No treatment-related clinical signs were observed. No treatment-related effects on body weight were observed. Macroscopic examination revealed no treatment-related abnormalities. Microscopic examination revealed dose-related incidences of squamous cell hyperplasia at or near the limiting ridge of the forestomach. **No adverse effects.** NOEL (M)=73.1 mg/kg/day (1000 ppm) and (F)=80.4 mg/kg/day (1000 ppm) (based on microscopic findings). **Acceptable.** (Corlett, 2/15/00)

015; 166670; "Thirteen Week Feeding Toxicity Study in Mice with BX-112 Technical" (Inoue, H., Biosafety Research Center, Food, Drugs, and Pesticides (An-Pyo Center), Shizuoka-ken, Japan, An-Pyo Center Report No. 2013, BASF Registration Document No. 91/11375, 5/1/91). BX-112 Technical (Lot No. G14-03, purity=92.1%) was admixed to the feed at dose levels of 0, 400, 2000, 10000, or 50000 ppm (0, 80, 389, 1986, and 10244 mg/kg/day, respectively, for males and 0, 93, 454, 2256, and 11916 mg/kg/day, respectively, for females) and fed to 10 B6C3F1 (C57BL/6XC3H) mice per sex per dose level

for 13 weeks. No animals died. No treatment-related clinical signs were observed. No treatment-related body weight or food efficiency effects were observed. Hematology revealed no treatment-related effects. Macroscopic and microscopic examinations revealed no treatment-related abnormalities. **No adverse effects indicated.** NOEL (M)=10244 mg/kg/day (50000 ppm), NOEL (F)=11916 mg/kg/day (50000 ppm) both based on no effects at HDT. **Supplemental** (no ophthalmological examinations conducted and no serum chemistry conducted). (Corlett, 2/23/00)

016; 166671; "BX-112: Preliminary Oral Toxicity Study in Beagle Dogs (Final Report-Repeated Daily Dosage for 4 Weeks)" (Massey, J.E. et al., Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, England, HRC Report No. KIC 29/89281, BASF Registration Document No. 89/10341, 2/3/97). BX-112 Technical (Lot Nos. G14-04 (purity=89.9%) and G14-05 (purity=92.3%)) was administered in gelatine capsules once per day to 2 pure-bred beagle dogs per sex per dose at dose levels of 0 (empty capsules), 200, 600, or 2000 mg/kg/day for 4 weeks. No animals died. Treatment-related pale and/or yellow colored feces were observed in all treatment groups. A treatment-related decrease in mean body weight gain (males and females combined) was observed at 2000 mg/kg/day. Hematology and serum chemistry revealed no treatment-related effects. No dose-related effects were observed on organ weights. Macroscopic and microscopic examinations revealed no treatment-related abnormalities. **No adverse effects indicated.** NOEL (M/F) not determined. **Supplemental** (no ophthalmological examinations conducted, only 2 animals per sex per dose used, and animals dosed for only 4 weeks). (Corlett, 2/25/00)

016; 166672; "BX-112: Oral Toxicity Study in Beagle Dogs (Final Report-Repeated Daily Dosage for 13 Weeks)" (Horner, S.A. et al., Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, England, HRC Report No. KIC 30/90433, BASF Registration Document No. 90/10501, 2/3/97). Technical BX-112 (Lot No. G14-09, purity=93.3%) was administered in gelatin capsules once per day to 4 pure-bred beagle dogs per sex per dose at dose levels of 0 (empty capsules), 80, 400, or 2000 mg/kg/day for 13 weeks. No animals died. A statistically significant decrease in potassium concentration was observed in both sexes at 400 and 2000 mg/kg/day. A dose-related increase in urine volume and a dose-related decrease in the specific gravity of the urine were observed in females at 2000 mg/kg/day. Microscopic examination revealed treatment-related dilated basophilic tubules in the cortex of the kidneys in both sexes at 2000 mg/kg/day. **No adverse effects.** NOEL (M/F)=80 mg/kg/day (based on a statistically significant decrease in potassium concentration and abnormal histological findings in the kidneys). **Acceptable.** (Corlett, 3/1/00)

METABOLISM STUDIES

** 026; 166696; "BX-112: Absorption, Distribution, Metabolism and Excretion Study in the Rat. Final Report: Volume 1 – Absorption, Distribution and Excretion"; (D. Hallifax; Pharmaco LSR Ltd, Eye, Suffolk IP23 7PX, England; Report No. 92/KCI113/0465; 5/4/93); Male and female Fisher 344 rats were dosed by gavage with 50 or 500 mg/kg of [^{14}C]-BX-112 (radiolabel at the 3 or 5 position on the cyclohexene ring)(lot no. CP-1107, radiochemical purity: 98.9%, specific activity: 97.8 µCi/mg). In Groups B (50 mg/kg) and D (500 mg/kg) (the mass balance studies), urine, feces and cage wash samples were collected up to 7 days from 5 animals/sex/group. In Group C1, 5 animals/sex were pretreated for 14 days with unlabelled BX-112 technical (purity: 93.8%), followed by 50 mg/kg of the radiolabelled material. Urine, feces and cage wash samples were collected up to 7 days after the final dose. In Groups B2 (50 mg/kg) and

D2 (500 mg/kg), blood was drawn periodically up to 96 hours after dosing for a pharmacokinetic analysis. A tissue and organ distribution study was performed by dosing 20 animals/sex/group in Groups B3 (50 mg/kg) and D3 (500 mg/kg) and determining the content of radiolabelled material in selected tissues and organs at 0.5, 3, 6 and 96 hours after dosing. A single dose biliary excretion study was performed in which the bile duct of 4 animals/sex was cannulated and the animals were treated with 50 mg/kg of the radiolabelled material. Bile, urine, feces, and cage wash samples were collected at selected intervals over the 24 hours post-dose. At both of the dosing levels, 94.2 to 97.3% of the total recovered dose, was excreted in the first 24 hours. Urine was the predominant site of recovery for the 50 mg/kg treatment group ((M) 58.3%, (F) 54.5% of the administered dose). In contrast, the feces were the primary site of recovery for the 500 mg/kg group ((M) 60.9%, (F) 58.2% of the administered dose). In both treatment groups, recovery in the cage wash constituted 21 to 25% of the total radioactivity administered. Repeated dosing for 14 days did not result in an altered excretion pattern. The pharmacokinetic parameters determined for both treatment levels were comparable (based upon the plasma analyses rather than the whole blood). In the tissue and organ distribution study, a greater percentage of the administered dose was recovered from the gastrointestinal tract of the high dose group in comparison with that of the low dose animals. The biliary excretion study confirmed that a very minimal amount of the radiolabelled material was excreted back into the feces via the bile duct. Absorption from the gastrointestinal tract was the limiting factor in the uptake of the test material. **Study acceptable.** (Moore, 3/3/00)

027, 028; 166697, 166698, 166699; "BX-112: Absorption, Distribution, Metabolism and Excretion Study in the Rat. Final Report: Volume 2 – Metabolism; Addendum to Final Report; BX-112: Idenfification of Major Metabolite in the Rat"; (D. Hallifax, J. O'Connor; Pharmaco LSR Ltd, Eye, Suffolk IP23 7PX, England; Report No. 92/KCI113/0465, Addendum: Report No. 93/KCI113/0695, Report No. 95/KCI155/0210; 5/28/93, Addendum: 8/5/94, 6/23/95); Male and female Fisher 344 rats were dosed by gavage with 50 or 500 mg/kg of [^{14}C]-BX-112 (radiolabel at the 3 or 5 position on the cyclohexene ring)(lot no. CP-1107, radiochemical purity: 98.9%, specific activity: 97.8 µCi/mg). In Groups B (50 mg/kg) and D (500 mg/kg) (the mass balance studies), urine, feces and cage wash samples were collected up to 7 days from 5 animals/sex/group. In Group C1, 5 animals/sex were pretreated for 14 days with unlabelled BX-112 technical (purity: 93.8%), followed by 50 mg/kg of the radiolabelled material. Urine, feces and cage wash samples were collected up to 7 days after the final dose. Samples collected between 0 and 48 hours after dosing were analyzed to determine specific metabolites. In addition, radiolabelled material was extracted from liver and kidney tissue of one male each treated with either 50 mg/kg (Group B3) and 500 mg/kg (Group D3) and euthanized at 0.5 hours after dosing. The test material was largely excreted in the urine and the feces as the free acid (50 mg/kg: 38.3 to 39.1%, 500 mg/kg: 60.9 to 68.0%, and 50 mg/kg, repeated dose: 44.2 to 53.7% of the adminstered dose). An additional compound isolated in the urine was tentatively identified as an ester glucuronide of BX-112. The total conjugated fraction of the test material constituted 20.4 and 21.7% in the 50 mg/kg group, 7.3 and 3.7% in the 500 mg/kg group and 23.2 and 17.7% of the administered dose in the 50 mg/kg, repeated dosing group for the males and females, respectively. The only other identified metabolite was recovered from the organic phase of the feces extraction. This material was the despropionyl free acid of BX-112. It constituted no more than 2.3% of the administered dose in any of the groups examined. In the liver and the kidney, the predominant material recovered at both of the dosing levels was the free acid of the BX-112 (liver: 81.2 and 93.1% of the recovered radiolabel in the 50 and 500 mg/kg, respectively; kidney: 70.1 and 91.8% of the recovered label in the 50 and 500 mg/kg groups, respectively); in the addendum, two of the previously unknown compounds were noted to be methyl esters of the test material and the despropionyl metabolite and were considered to be artifacts; further information was provided regarding the isolation of radiolabelled compounds in the feces; the isolated compound was largely the free acid of the parent compound as had been originally reported; in addition, use of a C18 HPLC column rather than the C8 one resulted in a different ratio of the parent compound and the conjugate noted in the original analysis; further analysis of this "conjugate" by FAB-MS and Atmospheric Pressure Electrospray HPLC/MS did not result in a definitive identification of the compound. **Supplemental Study.** (Moore, 3/6/00)